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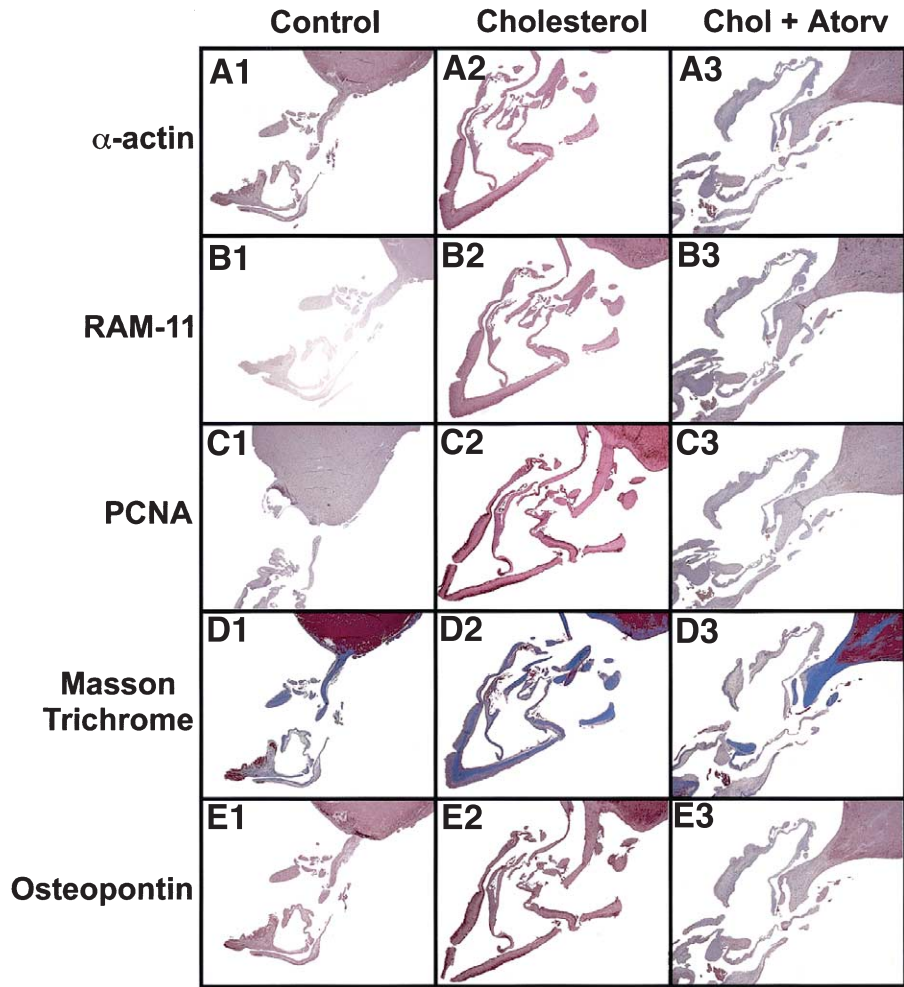
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Atorvastatin Decreases Cellular Proliferation and Bone Matrix Expression in the Hypercholesterolemic Mitral Valve

**To the Editor:** Idiopathic calcification of the mitral annulus is one of the most common cardiac abnormalities demonstrated at autopsy (1). Currently, the pathophysiology of mitral annular calcification (MAC) is viewed as a passive degenerative process due to a buildup of calcium along the mitral valve leaflet and annulus. Recent epidemiologic studies have defined the risk factors for the

development of MAC, which include hypertension, hypercholesterolemia, smoking, and male gender (2–4). These reports also have examined the association between mitral valve calcification and atherosclerosis; however, few experimental models have demonstrated these changes. Atherosclerosis is established as an active, cellular process within the vessel wall activated by an inflammatory



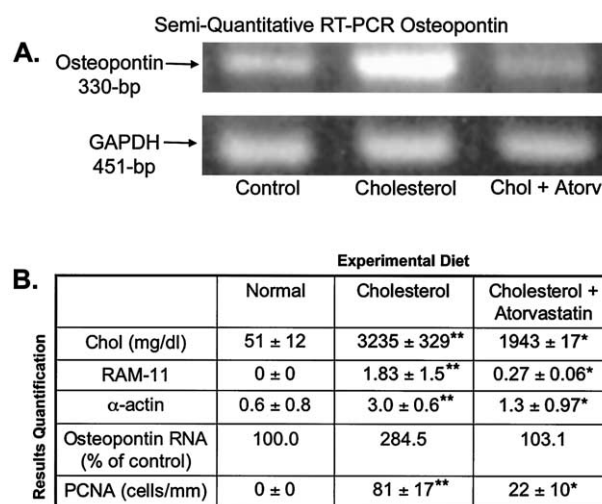
**Figure 1.** Light microscopy of rabbit mitral valves and papillary muscle. **Left column** = control diet; **middle column** = cholesterol diet with the **arrow** pointing to the valve leaflet; **right column** = cholesterol diet plus atorvastatin. (All frames: magnification,  $\times 12$ .) (A) Alpha-actin immunostain. (B) RAM-11, macrophage immunostain. (C) Proliferating cell nuclear antigen (PCNA) immunostain. (D) Masson trichrome stain. (E) Osteopontin immunostain.

mechanism (5). The mitral annulus and aortic valve cusps follow the coronary arteries as the most common sites of cardiac calcifications (6,7). Our laboratory has shown that experimental hypercholesterolemia induces an atherosclerotic aortic valve lesion that expresses bone matrix proteins that are modified by atorvastatin (8). In the present study, we examined experimental hypercholesterolemia with and without atorvastatin in rabbits and characterized the mitral valves.

All experiments were performed in accordance with the recommendations of the American Association for the Accreditation of Laboratory Animal Care (IACUC A282-99). Male New Zealand white rabbits weighing 2.5 to 3.0 kg were assigned to a control diet (n = 16), 1.0% cholesterol-fed diet (n = 16), or cholesterol- and atorvastatin-fed diet (n = 16) for eight weeks. Cholesterol-fed animals received a diet supplemented with 1.0% cholesterol (wt/wt; Purina Mills, Woodmont, Indiana), and the cholesterol- and atorvastatin-fed group was given atorvastatin at 2.5 mg/kg/day. Lipid levels were obtained. The mitral valves were harvested and embedded in paraffin and stored at  $-80^{\circ}\text{C}$  for RNA analysis. Immunohistochemistry was performed for the atherosclerotic markers alpha-actin, proliferating cell nuclear antigen, and macrophage (RAM11). Semiquantitative reverse-transcription polymerase chain reaction for osteopontin (OP) was performed. All experimental studies were performed as described previously (8). The samples were scored semiquantitatively by two observers who were blinded to the treatment arms, and the results were expressed qualitatively and demonstrated in the photomicrographs. No systematic differences existed between readers in the grading of stains by paired *t* tests ( $p = 0.33$ ). The kappa value for agreement between readers was 0.58, indicating moderate agreement. Comparison was made among the three groups using analysis of variance. The Scheffe method of adjustment was performed for multiple pairwise comparisons. All statistical tests were two-tailed, and  $p < 0.05$  was considered significant. The data are reported as the mean and the standard error of the mean.

The normal mitral valve in Figure 1, panel A1 shows a thin, intact valve attached to the papillary muscle as demonstrated by the alpha-actin immunostain. Furthermore, there was no evidence of foam cell formation (Fig. 1, B1), cellular proliferation (Fig. 1, C1), calcification shown by Masson trichrome staining (Fig. 1, D1), or OP deposition (Fig. 1, E1). However, mitral valves from the hypercholesterolemic showed a considerable increase in connective tissue and collagen formation (Fig. 1, D2). Likewise, there were focal areas of increased myofibroblast proliferating cell nuclear antigen staining and alpha-actin-positive staining cells (Fig. 1, A2 and C2), as well as areas staining positive for macrophages (RAM11), suggesting foam cell infiltration (Fig. 1, B2). Finally, there was significant deposition of OP within the mitral valve leaflet, which was not observed in the controls (Fig. 1, E2). Figure 1, panels A3 to E3 demonstrate the effects of the atorvastatin on the mitral valve for all of these treatments with marked improvement in the atherosclerotic lesion. We further confirmed the OP RNA expression in Figure 2A. There were significantly increased levels of OP RNA gene expression in the hypercholesterolemic animals compared with either the control or atorvastatin-treated animals. Figure 2B, demonstrates significant decreases in the amount of all the immunohistochemical markers with atorvastatin treatment.

It is evident from this experimental model that mitral valve calcification represent an active, complex process involving myofibroblast proliferation and osteoblast bone matrix protein expression and that this process may be modified by atorvastatin. There



**Figure 2.** Reverse transcription polymerase chain reaction (RT-PCR) and quantification data. (A) RT-PCR using the total ribonucleic acid (RNA) from the aortic valves for osteopontin (330 bp) results normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (451 bp). (B) Quantification results for cholesterol levels, RAM-11, alpha-actin, osteopontin, proliferating cell nuclear antigen (PCNA), and osteopontin RNA results. \*\* $p < 0.001$  compared with control; \* $p < 0.001$  compared with high-cholesterol diet.

have been numerous studies that correlate MAC and atherosclerotic disease. The clinical significance of MAC includes increased development of aortic atheromas, stroke, and peripheral vascular disease (9–11). These findings suggest that MAC should be added to other conventional risk factors for predicting coronary artery disease, as it may indicate a generalized atherosclerotic process within the valves and vasculature. The appearance of mitral valve calcifications may indicate a generalized atherosclerotic process and be predictive of coronary artery disease, suggesting that it should be included with other risk factors for coronary artery disease. Atorvastatin treatment reduces the cellular proliferation and early bone matrix expression, which may have implications for future treatment of patients in the early stages of MAC. The limitation of this study is the effect demonstrated in this study was found throughout the valve leaflet, including the mitral annulus. Future studies, including longer duration of cholesterol diet, are needed to demonstrate calcification in the future.

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Please note: Dr. Rajamannan is an inventor on a patent assigned to the Mayo Clinic titled “Method for Slowing Heart Valve Degeneration.” The Mayo Clinic owns all rights to the patent.

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Letters to the Editor

Cardiac Resynchronization Therapy, Central Sleep Apnea, and Cheyne-Stokes Respiration in Chronic Heart Failure Patients

We congratulate Sinha et al. (1) for their interesting and innovative report in JACC on the therapeutic effect of biventricular pacing on central sleep apnea (CSA). A few questions remain: the title suggests some information about Cheyne-Stokes respiration (CSR), which is not equivalent to CSA; however, results are lacking. Were all apnea/hypopnea events central in nature or did some also have obstructive or mixed events? Completely blinded serial scoring is difficult in patients undergoing an intervention such as an implantation device, and therefore a bias in visual scoring might be possible. We would be interested in learning some additional objective values such as desaturation index or time in apnea. This would be of greater value than minimum SaO<sub>2</sub> in their Table 3.

In their discussion, the investigators state that CSA is related to heart failure, a statement that contrasts to similar findings on exercise and echocardiography in both groups. In view of this, how do the researchers explain the dramatic effects of cardiac resynchronization therapy (CRT) on CSA when the benefit of CRT (on

echocardiography and exercise) is equal in patients with and without CSA? Moreover, the effect of CRT on sleep apnea, as described by Sinha et al. (1), is huge and much higher than in our experience and even higher than after cardiac transplantation (2,3). In these reports, cyclic respiration/CSR was abolished, but sleep apnea persisted or got worse in a considerable number of patients.

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REPLY

We thank Dr. Scharf and colleagues for their valuable comments to our study on central sleep apnea (CSA) and cardiac resynchronization therapy (CRT) (1). Importantly, all patients in our study had CSA and no evidence of obstructive sleep apnea (OSA). Because apnea occurred with a periodic pattern in patients with known advanced heart failure we believe that the breathing pattern can be classified as CSA although timing of arousals relative to respiratory events could not be evaluated with the polygraphy system used. We agree that desaturation index, breathing cycle length, and time in apnea add important information and have summarized these data in Table 1.

We have now studied more patients with mixed apnea or OSA and found less improvement in mixed apnea and no significant effect in OSA (2). Therefore, we believe that CRT works mainly on CSA by improving cardiac hemodynamics.

The notion that CSA is related to the severity of heart failure was derived from previous studies (3) and not from our data. We caution that our results refer to a small group of patients with short

**Table 1.** Additional Parameters Obtained by Polygraphy Before and During CRT

	Pre-CRT	On CRT	p Value
Desaturation index	23 ± 10.6	9.6 ± 5	0.002
Cycle length (s)	60.6 ± 5.7	45.8 ± 2.2	0.002
Ventilation/apnea length ratio	1.15 ± 0.12	0.42 ± 0.1	<0.001

CRT = cardiac resynchronization therapy.